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IS 12365 (1988): Method for determination of chlorpyrifos residues in food commodities [FAD 1: Pesticides and Pesticides Residue Analysis]



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“Knowledge is such a treasure which cannot be stolen”

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Indian Standard

METHOD FOR DETERMINATION OF CHLORPYRIFOS RESIDUES IN FOOD COMMODITIES

1. Scope

1.1 This standard prescribes a gas chromatographic method for the determination of chlorpyrifos [*O, O*-diethyl *O*-(3, 5, 6-trichloro-2-pyridyl)-phosphorothioate] residues in food commodities.

1.1.1 The method has a limit of detection of 0.005 $\mu\text{g/g}$ (0.005 ppm).

1.1.2 Standardized thin layer chromatographic (TLC) procedures may be followed, if necessary, for the confirmation of identity of the residues.

2. Sampling

2.1 The representative sample for the purpose of estimating chlorpyrifos residues in food commodities shall be in accordance with the sampling procedures as prescribed in IS : 11380 (Part 1)-1985 'Methods of sampling for the determination of pesticides residues: Part 1 Agricultural and food commodities'.

2.2 Preparation of the Laboratory Sample

2.2.1 Cereals, pulses, spices, condiments, tea, coffee, sugar, etc — If needed, grind to pass through 750 μ (20 mesh) sieve. Reduce to about 100 g by mixing and quartering.

2.2.2 Vegetables and fruits

- Remove visible soil particles by gentle rubbing. Root vegetables may need scrubbing with a brush and some washing.
- Remove all inedible portions of vegetables, such as outer leaves of cabbage, carrot tops, stems of tomatoes, stems and stones of fruits, etc.
- If a peel of skin is not normally consumed, it should be removed (for example, peas, oranges) and if edible, then it should be included (for example, carrots, potatoes).
- Cut a representative sample into pieces and reduce by mixing and quartering to about 300 g.
- Blend the 300 g sample in a waring blender to obtain homogeneous mixture. Mix contents with spatula and reblend to ensure homogeneity.

2.2.3 Oilseeds and nuts

- Remove and discard shells, if present.
- Reduce the sample by mixing and quartering to the desired sample size needed for extraction. Chop finely.

2.2.4 Fats as butter, ghee, cream, etc

- Melt the sample and filter to separate oil from other solids which may be present.
- Store the sample in a refrigerator.
- Cut the sample into small cubes.
- Place cubes (weighing 25 g) in a beaker and heat in an oven at 50°C for the oil to separate.

2.2.5 Vegetable oils (hydrogenated and unhydrogenated) — These do not require any processing.

2.2.6 Milk and curd — Check the homogeneity of the sample. If it is not homogeneous, shake gently.

2.2.7 Cheese — Chop finely and reduce the sample by mixing and quartering to the desired sample size.

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2.2.8 Meats

- a) Remove the inedible portions like head, scales, bones, etc.
- b) Homogenize the composite sample in a waring blender.

2.2.9 Eggs — Discard shells and mix yolks and whites.

2.2.10 Water and beverages — These do not require any processing.

2.3 Storage of Samples — Store samples as such, or, if the bulk is too much, after preparation of the laboratory sample. Depending upon the nature of the sample and the duration of storage, keep the samples or their extracts either in deep-freezer at -15°C or in refrigerator until taken up for analysis. Ensure that the samples do not absorb or lose moisture during storage. Avoid undue long storage periods.

3. Apparatus and Reagents

3.1 Blender

3.2 Rotary Vacuum Evaporator

3.3 Chromatographic Column — Glass, 40×1.5 cm with a 100 ml capacity reservoir and stop-cock arrangement.

3.4 Acetone — glass distilled, analytical reagent grade.

3.5 *n*-Hexane — glass distilled, analytical reagent grade.

3.6 Silica Gel — (60-100 mesh) activated at 110°C for 1 hour.

3.7 Sodium Sulphate (Anhydrous) — analytical reagent grade.

3.8 Acetonitrile (Methyl Cyanide) — glass distilled.

3.9 Ether — glass distilled.

3.10 Ethyl Acetate — glass distilled.

4. Procedure

4.0 If some sample extracts are difficult to filter (for example, wheat flour, rice, bananas) or there is emulsion formation during partitioning, obtain a clear solution by centrifuging at 3 000 revolutions per minute for 10 minutes or more.

4.1 Extraction

4.1.1 Non-fatty foods — (with less than 2 percent fat content)

4.1.1.1 Commodities with more than 75 percent moisture and less than 5 percent sugar content (most of the vegetables and some fruits), extract 50 g of the finely chopped representative sample twice with 200 and 100 ml of acetone and 50 g of anhydrous sodium sulphate by blending for 2 minutes each time. Alternatively, the sample can be macerated in the blender and extracted twice with 200 and 100 ml of the solvent by shaking for 30 and 15 minutes in an end-over-end shaker or horizontal soil shaking machine. Filter the extracts through buchner funnel using Whatman No. 1 or equivalent filter paper under suction. Rinse twice with 25 ml of acetone and concentrate in rotary vacuum evaporator over a water bath at 50°C to about 50 ml.

4.1.1.2 Commodities with less than 75 percent moisture (for example, cereals, pulses), extract 50 g of the sample with 200 ml acetone-water (8 : 2) without sodium sulphate and proceed as in 4.1.1.1.

4.1.1.3 Water and beverages — Partition 1 litre of the representative sample thrice with 100, 100, 50 ml hexane-ether (9:1). Dry the combined extracts through anhydrous sodium sulphate and concentrate in rotary vacuum evaporator over a water bath at 50°C and take in suitable volume of hexane for gas chromatography.

4.1.2 Fatty foods — with more than 2 percent fat content.

4.1.2.1 Oil and fats — Dissolve 10 g of the sample in 100 ml of hexane and partition twice into 125 ml of hexane-saturated acetonitrile, each time collecting the lower layer. Concentrate the combined acetonitrile layer to about 50 ml.

4.1.2.2 Milk — Take 100 ml in the blender, add 100 ml each of acetone and *n*-hexane and blend for 2 minutes. Transfer the material into centrifuge bottles and centrifuge 2 000 revolutions

per minute for 10 minutes. Take upper organic phase in a 500-ml separatory funnel and re-extract the lower layer twice with 100 ml portions of *n*-hexane and collect as before. Wash the combined *n*-hexane twice with 200 ml portions of distilled water. Dry the combined upper *n*-hexane phase over anhydrous sodium sulphate and concentrate to about 100 ml on rotary vacuum evaporator.

4.1.2.3 Meat, eggs, nuts, oilseeds — With a finely powdered sample containing about 5 g of fat, extract as before with 150 and 100 ml acetonitrile. Filter and rinse twice with 25 ml of the solvent. Concentrate the combined extract in a rotary vacuum evaporator over a water bath (less than 50°C) to about 50 ml.

4.2 Liquid-Liquid Partitioning

4.2.1 For non-fatty foods (4.1.1) except for water and beverages — Take the concentrated extract in a 500 ml separatory funnel. Dilute with 250 ml of 5 percent aqueous sodium chloride and partition into 150, 150 and 100 ml hexane. Pass the combined hexane layers through anhydrous sodium sulphate and concentrate as before to near dryness and take in about 10 ml hexane for adsorption chromatography.

4.2.2 For fatty foods except for milk — Proceed as described in 4.2.1.

4.2.2 For milk — Partition the extract (4.1.2.2) twice into 125 ml of acetonitrile and concentrate the acetonitrile layer as before to about 50 ml. Dilute with 250 ml of 5 percent aqueous sodium chloride and partition into 150, 150 and 100 ml hexane, pass through anhydrous sodium sulphate and concentrate to near-dryness and take in about 10 ml hexane for adsorption chromatography.

4.3 Adsorption Chromatography (Column Clean Up) — Wet pack 5 g anhydrous sodium sulphate, 20 g of silica gel and 10 g anhydrous sodium sulphate bottom upward in the glass column and prewash with 50 ml hexane. Further concentrate the extracts after partitioning step (from 4.2.1 and 4.2.2) to about 5 ml using a gentle stream of nitrogen and add it to the column. Just as the extract drains into the sodium sulphate layer, add 150 ml of 5 percent ethyl acetate in hexane and elute chlorpyrifos. Concentrate as before, take in suitable volume of hexane for estimation by gas chromatography.

4.4 Gas Chromatography (GLC)

4.4.1 Gas chromatography — Equipped with flame photometric detector with phosphorus specific filter. Alternatively, a GLC with electron capture detector (ECD) may also be used but it is essential to run reagent blanks while standardizing the procedure as ECD warrants stringent clean up requirements. The operating parameters suggested may be varied according to the available facilities provided the standardization is done:

Column : Glass, 120 mm long, 3mm ID, packed with 5 percent DC-200, on 80-100 mesh gas chrom Q

Temperature:

Injection port : 250°C

Column : 190°C

Detector : 250°C

Gas flow:

Nitrogen : 50 ml/minute

Hydrogen : 1 kg/cm²

Air : 1 kg/cm²

4.4.2 Reagents — Working standard solutions of chlorpyrifos reference standard of known purity at the concentration of 0.1, 0.2, 0.5, 1.0, 2.0 µg/ml in *n*-hexane. Suitably adjust the instrument so as to get at least 50 percent full-scale deflection with a 1 µg injection.

4.4.3 Procedure — The volume of clean-up sample solution (4.3.3) is adjusted either by concentration or dilution with hexane to achieve on-scale peaks. Inject 2-5 µl of the sample and identify the presence of chlorpyrifos residues by comparing the retention time of the sample peak with that of the reference standard.

4.4.4 Calculation

$$\text{Chlorpyrifos residue (} \mu\text{g/g) } = \frac{A_1 \times M_1 \times V_1}{A_2 \times V_2 \times M_2} \times f$$

where

A_1 = peak height/area of the sample;

A_2 = peak height/area of the standard chlorpyrifos;

M_1 = mass of standard chlorpyrifos injected (μg);

M_2 = mass of sample taken for analysis;

V_1 = volume of sample in ml;

V_2 = volume of sample injected, in μl ; and

$$f = \text{Recovery factor} = \frac{100}{\text{percent mean recovery}}$$

Note — Percent mean recovery is determined by taking untreated control sample to which a known amount of chlorpyrifos is added and analysed as described above.

EXPLANATORY NOTE

Chlorpyrifos formulations are used in agriculture, public health and veterinary for the control of various insect pests. This standard will enable the health authorities and others engaged in the field to follow uniform test procedure for the estimation of chlorpyrifos residues in various food commodities.

In the preparation on this standard, due consideration has been given to the limits of chlorpyrifos residues which are likely to be laid down under the provisions of *Prevention of Food Adulteration Act, 1954* and rules framed thereunder.

In reporting the result of a test made in accordance with the standard, if the final value, observed or calculated, is to be rounded off, it shall be done in accordance with IS : 2-1960 'Rules for rounding off numerical values (revised)'.